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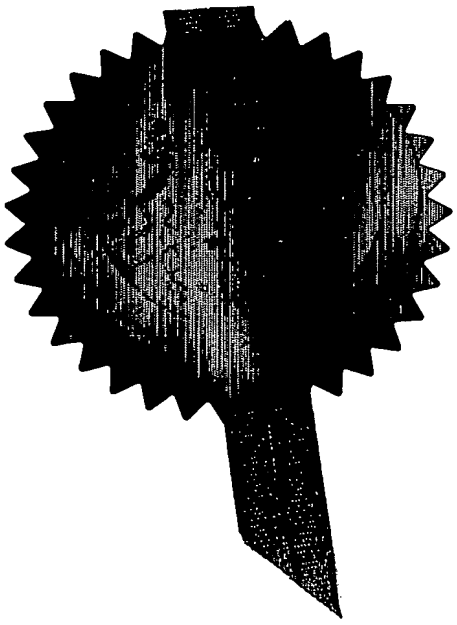

Signed A M Brewer.

Dated 17 December 2004

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1/77

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P01/7700 0.00-0327760.5

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

29 NOV 2003

The Patent Office

Cardiff Road
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1. Your reference 101305-1 GB

2. Patent application number *(The Patent Office will fill in this part)* 0327760.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca AB
SE-151 85 Sodertalje
Sweden

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation Sweden

7822448003

4. Title of the invention

COMPOUNDS

5. Name of your agent *(if you have one)*

Tracey BRYANT

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

AstraZeneca
Global Intellectual Property
PO Box 272
Mereside, Alderley Park
Macclesfield,
Cheshire SK10 4GR

Patents ADP number *(if you know it)*

8179707001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

COMPOUNDS

The present invention relates to a group of benzoyl amino pyridyl carboxylic acids which are useful in the treatment or prevention of a disease or medical condition mediated through glucokinase (GLK), leading to a decreased glucose threshold for insulin secretion. In addition the compounds are predicted to lower blood glucose by increasing hepatic glucose uptake. Such compounds may have utility in the treatment of Type 2 diabetes and obesity. The invention also relates to pharmaceutical compositions comprising said compounds and to methods of treatment of diseases mediated by GLK using said compounds.

In the pancreatic β-cell and liver parenchymal cells the main plasma membrane glucose transporter is GLUT2. Under physiological glucose concentrations the rate at which GLUT2 transports glucose across the membrane is not rate limiting to the overall rate of glucose uptake in these cells. The rate of glucose uptake is limited by the rate of phosphorylation of glucose to glucose-6-phosphate (G-6-P) which is catalysed by glucokinase (GLK) [1]. GLK has a high (6-10mM) Km for glucose and is not inhibited by physiological concentrations of G-6-P [1]. GLK expression is limited to a few tissues and cell types, most notably pancreatic β-cells and liver cells (hepatocytes) [1]. In these cells GLK activity is rate limiting for glucose utilisation and therefore regulates the extent of glucose induced insulin secretion and hepatic glycogen synthesis. These processes are critical in the maintenance of whole body glucose homeostasis and both are dysfunctional in diabetes [2].

In one sub-type of diabetes, Type 2 maturity-onset diabetes of the young (MODY-2), the diabetes is caused by GLK loss of function mutations [3, 4]. Hyperglycaemia in MODY-2 patients results from defective glucose utilisation in both the pancreas and liver [5]. Defective glucose utilisation in the pancreas of MODY-2 patients results in a raised threshold for glucose stimulated insulin secretion. Conversely, rare activating mutations of GLK reduce this threshold resulting in familial hyperinsulinism [6, 7]. In addition to the reduced GLK activity observed in MODY-2 diabetics, hepatic glucokinase activity is also decreased in type 2 diabetics [8]. Importantly, global or liver selective overexpression of GLK prevents or reverses the development of the diabetic phenotype in both dietary and genetic models of the disease [9-12]. Moreover, acute treatment of type 2 diabetics with fructose improves glucose tolerance through stimulation of hepatic glucose utilisation [13]. This effect is believed to be

In WO0058293 and WO01/44216 (Roche), a series of benzylcarbamoyl compounds are described as glucokinase activators. The mechanism by which such compounds activate GLK is assessed by measuring the direct effect of such compounds in an assay in which GLK activity is linked to NADH production, which in turn is measured optically - see details of the *in vitro* assay described in Example A. Compounds of the present invention may activate GLK directly or may activate GLK by inhibiting the interaction of GLKRP with GLK. The latter mechanism offers an important advantage over direct activators of GLK in that they will not cause the severe hypoglycaemic episodes predicted after direct stimulation. Many compounds of the present invention may show favourable selectivity compared to known GLK activators.

WO9622282, WO9622293, WO9622294, WO9622295, WO9749707 and WO9749708 disclose a number of intermediates used in the preparation of compounds useful as vasopressin agents which are structurally similar to those disclosed in the present invention. Structurally similar compounds are also disclosed in WO9641795 and JP8143565 (vasopressin antagonism), in JP8301760 (skin damage prevention) and in EP619116 (osetopathy).

WO01/12621 describes the preparation of as isoxazolylpyrimidines and related compounds as inhibitors of c-JUN N-terminal kinases, and pharmaceutical compositions containing such compounds.

Cushman *et al* [Bioorg Med Chem Lett (1991) 1(4), 211-14] describe the synthesis of pyridine-containing stilbenes and amides and their evaluation as protein-tyrosine kinase inhibitors. Rogers *et al* [J Med Chem (1981) 24(11) 1284-7] describe mesoionic purinone analogs as inhibitors of cyclic-AMP phosphodiesterase.

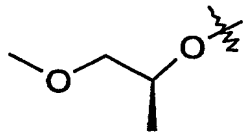
WO00/26202 describes the preparation of 2-amino-thiazole derivatives as antitumour agents. GB 2331748 describes the preparation of insecticidal thiazole derivatives. WO96/36619 describes the preparation of aminothiazole derivatives as ameliorating agents for digestive tract movements. US 5466715 and US 5258407 describe the preparation of 3,4-disubstituted phenol immunostimulants. JP 58069812 describes hypoglycemic pharmaceuticals containing benzamide derivatives. US 3950351 describes 2-benzamido-5-nitrothiazoles and Cavier *et al* [Eur J Med Chem – Chim Ther (1978) 13(6), 539-43] discuss the biological interest of these compounds.

Pending International application Number: PCT/GB02/02873 describes a group of benzoyl amino pyridyl carboxylic acids which are activators of the enzyme glucokinase (GLK). We have surprisingly found a small selection of these compounds which have a

compounds may exist in tautomeric forms and that the invention also relates to any and all tautomeric forms of the compounds of the invention which activate GLK.

Preferred compounds of Formula (I) are those wherein any one or more of the following apply:

(1) The group at the 3 position in Formula (I) is:



;

(2) R^2 is hydrogen;

(3) R^2 is fluoro or chloro;

(4) R^2 is methyl

(5) R^2 is fluoro;

(6) R^2 is chloro;

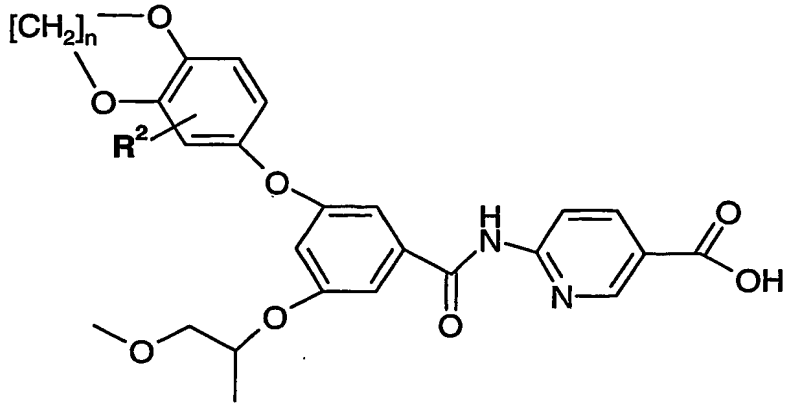
(7) n is 1;

(8) n is 2;

(7) R^2 is linked to the phenyl ring to which it is attached at the 3-position relative to the oxygen atom.

According to a further feature of the invention there is provided the following preferred groups of compounds of the invention:

(I) a compound of Formula (Ia)

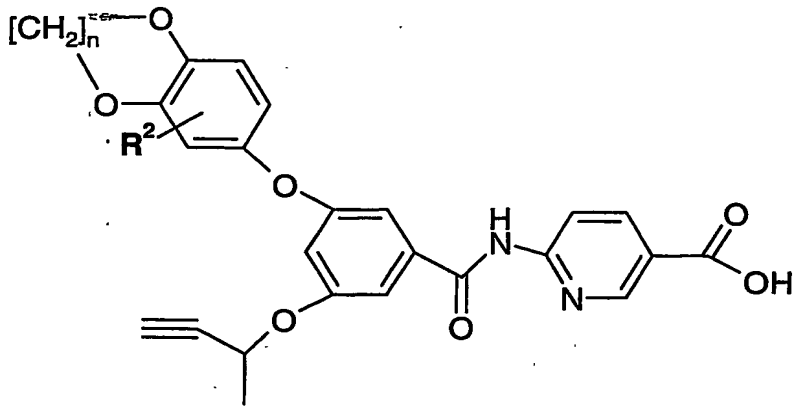


Formula (Ia)

wherein:

n and R^2 are as defined above in a compound of Formula (I);

(IV) a compound of Formula (Id)



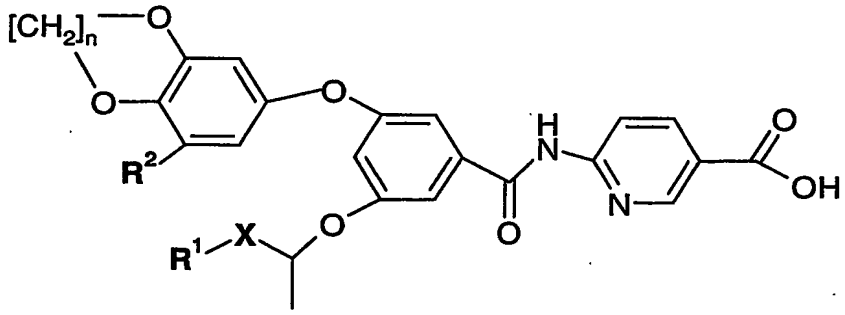
Formula (Id)

wherein:

n and $\mathbf{R^2}$ are as defined above in a compound of Formula (I);

or a salt, solvate or pro-drug thereof.

(V) a compound of Formula (Id)



Formula (Ie)

wherein:

n, **X**, $\mathbf{R^1}$ and $\mathbf{R^2}$ are as defined above in a compound of Formula (I);

or a salt, solvate or pro-drug thereof.

Preferred compounds of the invention include:

6-{[(3-(2,3-dihydro-1,4-benzodioxin-6-yloxy)-5-{[(1S)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino}pyridine-3-carboxylic acid

6-{[(3-(1,3-benzodioxol-5-yloxy)-5-{[(1S)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino}pyridine-3-carboxylic acid

or a salt, solvate or pro-drug thereof.

The compounds of the invention may be administered in the form of a pro-drug. A pro-drug is a bioprecursor or pharmaceutically acceptable compound being degradable in

example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric or maleic acid. In addition a suitable pharmaceutically-acceptable salt of a benzoxazinone derivative of the invention which is sufficiently acidic is an alkali metal salt, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base which affords a physiologically-acceptable cation, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

A further feature of the invention is a pharmaceutical composition comprising a compound of Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie) as defined above, or a salt, solvate or prodrug thereof, together with a pharmaceutically-acceptable diluent or carrier.

According to another aspect of the invention there is provided a compound of Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie) as defined above for use as a medicament.

Further according to the invention there is provided a compound of Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie) for use in the preparation of a medicament for treatment of a disease mediated through GLK, in particular type 2 diabetes.

The compound is suitably formulated as a pharmaceutical composition for use in this way.

According to another aspect of the present invention there is provided a method of treating GLK mediated diseases, especially diabetes, by administering an effective amount of a compound of Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie), or salt, solvate or pro-drug thereof, to a mammal in need of such treatment.

Specific diseases which may be treated by a compound or composition of the invention include: blood glucose lowering in Diabetes Mellitus type 2 without a serious risk of hypoglycaemia (and potential to treat type 1), dyslipidemia, obesity, insulin resistance, metabolic syndrome X, impaired glucose tolerance.

As discussed above, thus the GLK/GLKRP system can be described as a potential "Diabesity" target (of benefit in both Diabetes and Obesity). Thus, according to another aspect of the invention there if provided the use of a compound of Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie), or salt, solvate or pro-drug thereof, in the preparation of a medicament for use in the combined treatment or prevention of diabetes and obesity.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate, anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a

Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

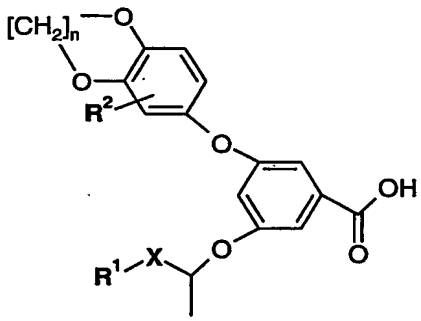
The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie) will naturally vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to well known principles of medicine.

In using a compound of the Formula (I), (Ia), (Ib), (Ic), (Id) or (Ie) for therapeutic or prophylactic purposes it will generally be administered so that a daily dose in the range, for example, 0.5 mg to 75 mg per kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.5 mg to 30 mg per kg body weight will generally be used. Similarly, for administration by inhalation, a dose in the range, for example, 0.5 mg to 25 mg per kg body weight will be used. Oral administration is however preferred.

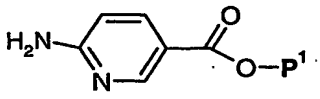
The elevation of GLK activity described herein may be applied as a sole therapy or in combination with one or more other substances and/or treatments for the indicated being treated. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. Simultaneous treatment may be in a single tablet or in separate tablets. For example in the treatment of diabetes mellitus, chemotherapy may include the following main categories of treatment:

1) Insulin and insulin analogues;
2) Insulin secretagogues including sulphonylureas (for example glibenclamide, glipizide) and prandial glucose regulators (for example repaglinide, nateglinide);
3) Insulin sensitising agents including PPARγ agonists (for example pioglitazone and rosiglitazone);
4) Agents that suppress hepatic glucose output (for example metformin).
5) Agents designed to reduce the absorption of glucose from the intestine (for example acarbose);
6) Agents designed to treat the complications of prolonged hyperglycaemia;
7) Anti-obesity agents (for example sibutramine and orlistat);
8) Anti- dyslipidaemia agents such as, HMG-CoA reductase inhibitors (statins, eg rosuvastatin, pravastatin); PPARα agonists (fibrates, eg gemfibrozil); bile acid

(a) reaction of an acid of Formula (IIIa) or activated derivative thereof with a compound of Formula (IIIb),



Formula (IIIa)

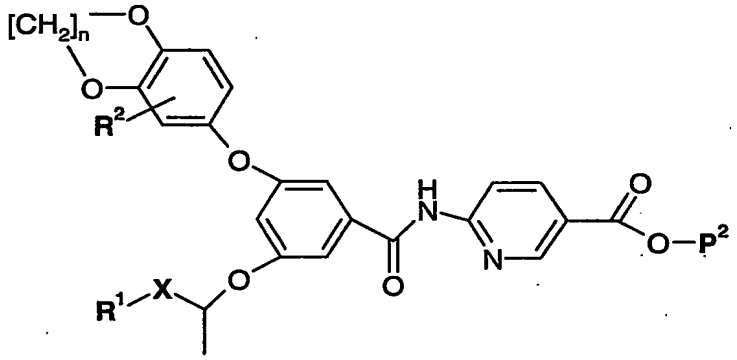


Formula (IIIb);

wherein $\mathbf{P^1}$ is hydrogen or a protecting group

or

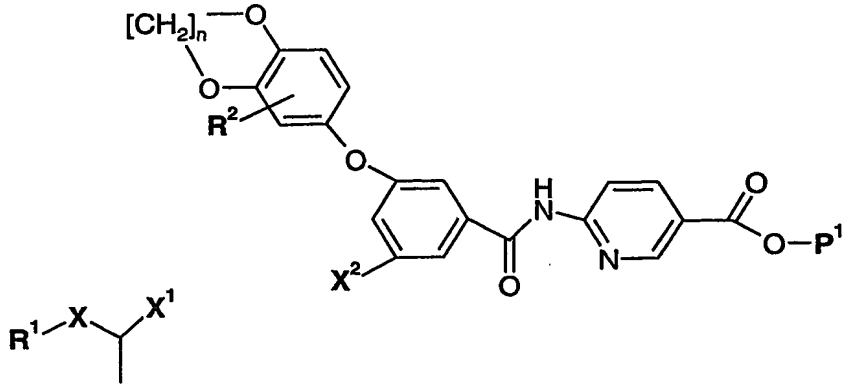
(b) de-protection of a compound of Formula (IIIc),



Formula (IIIc)

wherein $\mathbf{P^2}$ is a protecting group; or

(c) reaction of a compound of Formula (IIId) with a compound of Formula (IIIe),



Formula (IIId)

Formula (IIIe)

(i) using an appropriate coupling reaction, such as a carbodiimide coupling reaction performed with EDAC in the presence of DMAP in a suitable solvent such as DCM, chloroform or DMF at room temperature; or

(ii) reaction in which the carboxylic group is activated to an acid chloride by reaction with oxalyl chloride in the presence of a suitable solvent such as methylene chloride. The acid chloride can then be reacted with a compound of Formula IIIb in the presence of a base, such as triethylamine or pyridine, in a suitable solvent such as chloroform or DCM at a temperature between 0°C and room temperature.

Process b) – de-protection reactions are well know in the art. Examples of P^1 include C_{1-6}alkyl and benzyl. Wherein P^1 is an C_{1-6}alkyl, the reaction can be performed in the presence of sodium hydroxide in the suitable solvent such as THF/water.

Process c) – compounds of Formula (IIId) and (IIIe) can be reacted together in a suitable solvent, such as DMF or THF, with a base such as sodium hydride or potassium *tert*-butoxide, at a temperature in the range 0 to 100°C, optionally using metal catalysis such as palladium(II)acetate, palladium on carbon, copper(II)acetate or copper(I)iodide; Alternatively, compounds of Formula (IIId) and (IIIe) can be reacted together in a suitable solvent, such as THF or DCM, with a suitable phosphine such as triphenylphosphine, and azodicarboxylate such as diethylazodicarboxylate;

Process d) - compounds of Formula (IIId) and (IIIe) can be reacted together in a suitable solvent, such as DMF or THF, with a base such as sodium hydride or potassium tert-butoxide, at a temperature in the range 0 to 100°C, optionally using metal catalysis such as palladium(II)acetate, palladium on carbon, copper(II)acetate or copper(I)iodide;

Process e) – reaction of a compound of Formula (IIIh) with a compound of Formula (IIIi) can be performed in a polar solvent, such as DMF or a non-polar solvent such as THF with a strong base, such as sodium hydride or potassium *tert*-butoxide at a temperature between 0 and 100°C, optionally using metal catalysis, such as palladium(II)acetate, palladium on carbon, copper(II)acetate or copper(I)iodide.

During the preparation process, it may be advantageous to use a protecting group for a functional group within the molecule. Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

Methods appropriate for removal of hydroxy and amino protecting groups include, for example, acid-, base, metal- or enzymically-catalysed hydrolysis, or photolytically for groups such as o-nitrobenzyloxycarbonyl, or with fluoride ions for silyl groups.

Examples of protecting groups for amide groups include aralkoxymethyl (e.g. benzyloxymethyl and substituted benzyloxymethyl); alkoxymethyl (e.g. methoxymethyl and trimethylsilylethoxymethyl); tri alkyl/arylsilyl (e.g. trimethylsilyl, t-butyldimethylsily, t-butyldiphenylsilyl); tri alkyl/arylsilyloxymethyl (e.g. t-butyldimethylsilyloxymethyl, t-butyldiphenylsilyloxymethyl); 4-alkoxyphenyl (e.g. 4-methoxyphenyl); 2,4-di(alkoxy)phenyl (e.g. 2,4-dimethoxyphenyl); 4-alkoxybenzyl (e.g. 4-methoxybenzyl); 2,4-di(alkoxy)benzyl (e.g. 2,4-di(methoxy)benzyl); and alk-1-enyl (e.g. allyl, but-1-enyl and substituted vinyl e.g. 2-phenylvinyl).

Aralkoxymethyl, groups may be introduced onto the amide group by reacting the latter group with the appropriate aralkoxymethyl chloride, and removed by catalytic hydrogenation. Alkoxymethyl, tri alkyl/arylsilyl and tri alkyl/silyloxymethyl groups may be introduced by reacting the amide with the appropriate chloride and removing with acid; or in the case of the silyl containing groups, fluoride ions. The alkoxyphenyl and alkoxybenzyl groups are conveniently introduced by arylation or alkylation with an appropriate halide and removed by oxidation with ceric ammonium nitrate. Finally alk-1-enyl groups may be introduced by reacting the amide with the appropriate aldehyde and removed with acid.

The following examples are for illustration purposes and are not intended to limit the scope of this application. Each exemplified compound represents a particular and independent aspect of the invention. In the following non-limiting Examples, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in *vacuo* and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at room temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

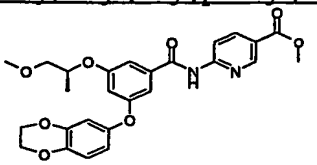
(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the Formula (I) were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are

solid was filtered, washed with water and dried to give 6-{[(3-(2,3-dihydro-1,4-benzodioxin-6-yloxy)-5-{[(1*S*)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino} pyridine-3-carboxylic acid.

m/z 481 $(M+H)^+$.

Methyl-6-{[(3-(2,3-dihydro-1,4-benzodioxin-6-yloxy)-5-{[(1*S*)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino}pyridine-3-carboxylate

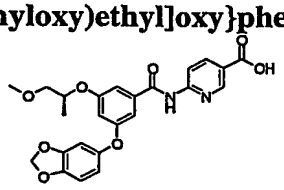


A solution of Methyl-6-{[3-hydroxy-5-{[(1*S*)-1-methyl-2-(methyloxy)ethyl]oxy} phenyl)carbonyl]amino}pyridine-3-carboxylate (0.75 mmol), 1,4-benzodioxin-6-boronic acid (0.75 mmol), copper (II) acetate (0.75mmol), triethylamine (3.75 mmol) and freshly activated 4A molecular sieves (1 g) in DCM (10 mL) was stirred at ambient temperature and under ambient atmosphere for 2 days. The reaction mixture was filtered, the DCM removed *in vacuo* and the residual oil partitioned between ethyl acetate and hydrochloric acid (1N). The ethyl acetate layer was separated, washed with aqueous sodium hydrogen carbonate solution, brine, dried ($MgSO_4$) and evaporated to a residue which was chromatographed on silica with 40% ethyl acetate in *iso*-hexane as eluant to give Methyl-6-{[(3-(2,3-dihydro-1,4-benzodioxin-6-yloxy)-5-{[(1*S*)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino} pyridine-3-carboxylate.

m/z 495 $(M+H)^+$. ^1H NMR ($CDCl_3$): 1.3 (d, 3H), 3.4 (s, 3H), 3.5 (m, 2H), 4.0 (s, 3H), 4.3 (s, 4H), 4.6 (m, 1H), 6.6 (m, 2H), 6.75 (m, 1H), 6.85 (dd, 1H), 7.0 (m, 1H), 7.2 (m, 1H), 8.3 (m, 2H), 8.7 (s, 1H), 8.95 (s, 1H).

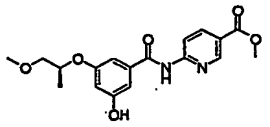
EXAMPLE 2

6-{[(3-(1,3-benzodioxol-5-yloxy)-5-{[(1*S*)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino}pyridine-3-carboxylic acid



appropriate bromide (10 mmol) in ether (25 mL) at –78°C was added a 1.6M solution of *n*-butyl lithium in hexane (11 mmol). The reaction mixture was stirred at –78°C for 10 minutes, tri-isopropyl borate (11mmol) added and the reaction mixture stirred at –78°C for 30 minutes. The reaction mixture was allowed to come to ambient temperature, stirred for a further 30 minutes then quenched with water (20 mL). The aqueous layer was separated, washed with ether (25 mL) and acidified to pH 1 with concentrated hydrochloric acid. The resulting solid was filtered off, washed with water and dried to give the desired boronic acid.

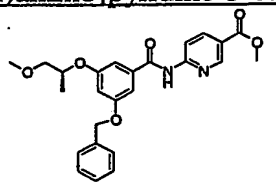
Methyl 6-{[(3-hydroxy-5-{[(*1S*)-1-methyl-2-(methyloxy)ethyl]oxy}phenyl)carbonyl]amino}pyridine-3-carboxylate



To a stirred solution of methyl 6-[({3-{[(*1S*)-1-methyl-2-(methyloxy)ethyl]oxy}-5-[(phenylmethyl)oxy]phenyl}carbonyl)amino]pyridine-3-carboxylate (0.038 mol) in THF (85 mL) was added methanol (85 mL). Palladium on charcoal catalyst (1.7g of 10% w/w) was added under an argon atmosphere, and the resulting suspension stirred at ambient temperature overnight in an atmosphere of hydrogen. The catalyst was filtered off through celite, washed with THF, and the filtrate evaporated to give a pale brown solid. This was triturated with ether to give the desired compound (72% yield).

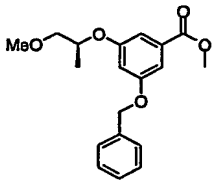
m/z 361 $(M+H)^+$, 359 $(M-H)^-$; ^1H NMR δ (d_6-DMSO): 1.25 (d, 3H), 3.3 (s, 3H), 3.45 (m, 2H), 3.85 (s, 3H), 4.65 (m, 1H), 6.55 (m, 1H), 6.95 (m, 1H), 7.1 (m, 1H), 8.3 (m, 2H), 8.9 (m, 1H), 11.0, (s, 1H).

Methyl 6-[({3-{[(*1S*)-1-methyl-2-(methyloxy)ethyl]oxy}-5-[(phenylmethyl)oxy]phenyl}carbonyl)amino]pyridine-3-carboxylate



To a stirred solution of 3-{[(*1S*)-1-methyl-2-(methyloxy)ethyl]oxy}-5-[(phenylmethyl)oxy]benzoic acid (75.9 mmol) in DCM (250 mL) containing DMF (1 mL), oxalyl chloride was added dropwise under argon (151.7 mmol), and the resulting solution stirred for 4 hours. The solution was then evaporated *in vacuo*, azeotroped with more DCM

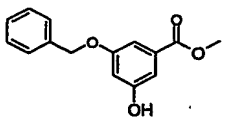
Methyl 3-{[(*1S*)-1-methyl-2-(methyloxy)ethyl]oxy}-5-[(phenylmethyl)oxy]benzoate



To a solution of methyl 3-hydroxy-5-[(phenylmethyl)oxy]benzoate (77.4 mmol) in THF was added polymer-supported triphenylphosphine (51.7g of 3 mmol/g loading, 155mmol) and (R)-(-)-1-methoxy-2-propanol (102 mmol). The stirred solution was blanketed with argon and cooled in an ice bath; a solution of diisopropyl azodicarboxylate (116 mmol) was added dropwise from a syringe over 10 minutes. After addition the solution was stirred for 20 minutes and then filtered, washing the residue with THF (500 mL); the filtrate and washings were combined and evaporated to give crude desired compound which was used in the next step without further purification.

^{1}H NMR δ (d_6-DMSO): 3.26 (s, 3H), 3.44 (m, 2H), 3.82 (s, 3H), 4.63 (m, 1H), 5.14 (s, 2H), 6.85 (s, 1H), 7.05 (s, 1H), 7.11 (s, 1H), 7.30-7.47 (m, 5H); the spectrum also contained signals consistent with a small amount of bis(1-methylethyl) hydrazine-1,2-dicarboxylate.

Methyl 3-hydroxy-5-[(phenylmethyl)oxy]benzoate



To a stirred solution of methyl 3,5-dihydroxybenzoate (5.95 mol) in DMF (6 L) was added potassium carbonate (9 mol), and the suspension stirred at ambient temperature under argon. To this was added benzyl bromide (8.42 mol) slowly over 1 hour, with a slight exotherm, and the reaction mixture stirred overnight at ambient temperature. It was then quenched cautiously with ammonium chloride solution (5 L) followed by water (35 L). The aqueous suspension was extracted with DCM (1x3 L and 2x5 L). The combined extracts were washed with water (10 L) and dried overnight ($MgSO_4$). The solution was evaporated in *vacuo*, and the crude product chromatographed in three batches (flash column, 3x2 kg silica, eluting with a gradient consisting of hexane containing 10% DCM, to neat DCM, to DCM containing 50% ethyl acetate) to eliminate starting material; the crude eluant was then chromatographed in 175 g batches (Amicon HPLC, 5 kg normal-phase silica, eluting with *iso*-hexane containing 20% v/v of ethyl acetate) to give the desired compound (21% yield).

Binding assays were performed at room temperature for 2 hours. The reaction mixtures contained 50mM Tris-HCl (pH 7.5), 2mM ATP, 5mM $MgCl_2$, 0.5mM DTT, recombinant biotinylated GLK (0.1 mg), recombinant GLKRP (0.1 mg), 0.05mCi [3H] F-6-P (Amersham) to give a final volume of 100ml. Following incubation, the extent of GLK/GLKRP complex formation was determined by addition of 0.1mg/well avidin linked SPA beads (Amersham) and scintillation counting on a Packard TopCount NXT.

(3) A F-6-P / GLKRP binding assay for measuring the binding interaction between GLKRP and F-6-P. This method may be used to provide further information on the mechanism of action of the compounds. Compounds identified in the GLK/GLKRP binding assay may modulate the interaction of GLK and GLKRP either by displacing F-6-P or by modifying the GLK/GLKRP interaction in some other way. For example, protein-protein interactions are generally known to occur by interactions through multiple binding sites. It is thus possible that a compound which modifies the interaction between GLK and GLKRP could act by binding to one or more of several different binding sites.

The F-6-P / GLKRP binding assay identifies only those compounds which modulate the interaction of GLK and GLKRP by displacing F-6-P from its binding site on GLKRP.

GLKRP is incubated with test compound and an inhibitory concentration of F-6-P, in the absence of GLK, and the extent of interaction between F-6-P and GLKRP is measured. Compounds which displace the binding of F-6-P to GLKRP may be detected by a change in the amount of GLKRP/F-6-P complex formed. A specific example of such a binding assay is described below

F-6-P / GLKRP scintillation proximity assay

Recombinant human GLKRP was used to develop a "mix and measure" 96 well scintillation proximity assay) as described in WO01/20327 (the contents of which are incorporated herein by reference). FLAG-tagged GLKRP is incubated with protein A coated SPA beads (Amersham) and an anti-FLAG antibody in the presence of an inhibitory concentration of radiolabelled [3H]F-6-P. A signal is generated. Compounds which displace the F-6-P will cause this signal to be lost. A combination of this assay and the GLK/GLKRP binding assay will allow the observer to identify compounds which disrupt the GLK/GLKRP binding interaction by displacing F-6-P.

Transformations

E. Coli transformations were generally carried out by electroporation. 400 ml cultures of strains DH5a or BL21(DE3) were grown in L-broth to an OD 600 of 0.5 and harvested by centrifugation at 2,000g. The cells were washed twice in ice-cold deionised water, resuspended in 1ml 10% glycerol and stored in aliquots at -70^{0}C. Ligation mixes were desalted using Millipore V series™ membranes (0.0025mm) pore size). 40ml of cells were incubated with 1ml of ligation mix or plasmid DNA on ice for 10 minutes in 0.2cm electroporation cuvettes, and then pulsed using a Gene Pulser™ apparatus (BioRad) at 0.5kVcm^{-1}, 250mF. Transformants were selected on L-agar supplemented with tetracyline at 10mg/ml or ampicillin at 100mg/ml.

Expression

GLK was expressed from the vector pTB375NBSE in E.coli BL21 cells,, producing a recombinant protein containing a 6-His tag immediately adjacent to the N-terminal methionine. Alternatively, another suitable vector is pET21(+)DNA, Novagen, Cat number 697703. The 6-His tag was used to allow purification of the recombinant protein on a column packed with nickel-nitrilotriacetic acid agarose purchased from Qiagen (cat no 30250).

GLKRP was expressed from the vector pFLAG CTC (IBI Kodak) in E.coli BL21 cells, producing a recombinant protein containing a C-terminal FLAG tag. The protein was purified initially by DEAE Sepharose ion exchange followed by utilisation of the FLAG tag for final purification on an M2 anti-FLAG immunoaffinity column purchased from Sigma-Aldrich (cat no. A1205).

Biotinylation of GLK:

GLK was biotinylated by reaction with biotinamidocaproate N-hydroxysuccinimide ester (biotin-NHS) purchased from Sigma-Aldrich (cat no. B2643). Briefly, free amino groups of the target protein (GLK) are reacted with biotin-NHS at a defined molar ratio forming stable amide bonds resulting in a product containing covalently bound biotin. Excess, non-conjugated biotin-NHS is removed from the product by dialysis. Specifically, 7.5mg of GLK was added to 0.31mg of biotin-NHS in 4mL of 25mM HEPES pH7.3, 0.15M KCl, 1mM dithiothreitol, 1mM EDTA, 1mM $MgCl_2$ (buffer A). This reaction mixture was dialysed

performed using a Phenomenex Prodigy C8, 50x4.6, 5μm.column (Phenomenex, Macclesfield, UK) at a flow rate of 1ml/minute using an injection volume of 10μl using the following gradient elution profile:

Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in methanol
Mobile phase gradient	0 min 50% A
	0.5 min 5% A
	2.5 min 5% A
	2.6 min 50% A
	3.0 min 50% A.

Mass spectroscopy was performed using an Applied Biosystems API3000 Mass spectrometer (Applied Biosystems, Foster City, California, USA). Prior to the running of samples the mass spectrometer was optimised for the structure of the test compound.

The concentration of test samples was determined from the ratio of the peak height of the test sample to the peak height of the internal standard. The concentration of the test sample was calculated with reference to a standard curve relating the ratio to the concentration prepared by using known concentrations of test sample added to samples of rat plasma using (3-isopropoxy-5-benzyoxy-benzoyl)amino pyridine 3-carboxylic acid as an internal standard, treated as described above.

Measurements of plasma protein binding of compounds

The plasma protein binding of compounds was measured using the equilibrium dialysis technique (W. Lindner et al, J.Chromatography, 1996, 677, 1-28). Compound was dialysed at a concentration of 20 μM for 18 hours at 37°C with plasma and isotonic phosphate buffer pH 7.4 (1ml of each in the dialysis cell). A Spectrum® 20-cell equilibrium dialyser was used together with Teflon, semi-micro dialysis cells and Spectra/Por®2 membrane discs with a molecular weight cut off 12-14000 Dalton, 47mm (supplied by PerBio Science UK Ltd, Tattenhall, Cheshire). Plasma and buffer samples are removed following dialysis and analysed using HPLCUV/MS (high performance liquid chromatography with UV and mass spec detection) to give the % free level in plasma.

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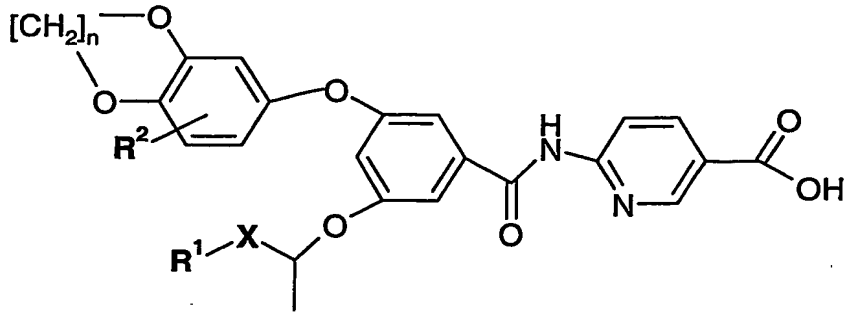
CLAIMS:

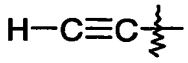
1. A compound of Formula (I):



Formula (I)

wherein:

R^1–X- is selected from: methyl, methoxymethyl or



;

R^2 is selected from hydrogen, methyl, chloro or fluoro;

n is 1 or 2;

or a salt, pro-drug or solvate thereof

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